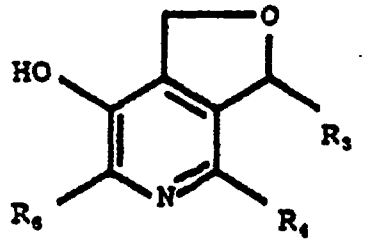
TITLE:

Separation of Isomers of Furo[3,4-c]Pyridine Derivatives

DESCRIPTION:

The invention relates to the separation of stereoisomers of furo[3,4-c]pyridine derivatives of the general formula I:



I.

wherein R_3 represents a hydrogen atom, a straight chain saturated hydrocarbon group having from 1 to 5 carbon atoms, an unsaturated hydroccarbon group having from 2 to 5 carbon atoms, a heterocyclic group having up to 6 ring atoms, a phenyl group, a phenylalkyl or phenylalkenyl group, said groups being optionally substituted by one or more chlorine or fluorine atoms, trifluoromethyl groups, alkyl groups having from 1 to 5 carbon atoms, alkoxy groups having from 1 to 5 carbon atoms, alkylthio groups having from 1 to 5 carbon atoms, dialkylamino groups in which each alkyl group has from 1 to 5 carbon atoms, dialkylaminoalkoxy groups in which each of the two alkyl groups and the alkoxy group has from 1 to 5 carbon atoms or α or β-alkoxy-N-pyrrolidinyl groups in which the alkoxy group has from 1 to 5 carbon atoms; R_4 represents a hydrogen or chlorine atom and R_6 represents a saturated or unsaturated hydrocarbon group up to C_5, optionally substituted by a hydroxy radical, by an amino residue of the formula $N(R)_2$ whereir R represents a hydrogen atom or an alkyl group up to C_3.

These compounds are known in their racemic form, for instance from our Patent Specifications GB 2092586, 2148292, 2149781, 2149782, 2153824 and 2164037. They have various therapeutic activities, but it has been

found that, for most of them one stereoisomer is more active than the other. It is thus desirable to devise a method for the separation of their stereoisomers.

The invention provides a method for the separation of stereoisomers of 7-hydroxy-furo[3,4-c]pyridine derivatives of the general formula I which method comprises

(a) reacting a fully O-acetylated monosaccharide halogenide with a racemate of the selected 7-hydroxyfuro[3,4-c]pyridine derivative to form the corresponding (+) and (-) (O-acetylated monosaccharide) (furo[3,4-c]pyridine-7-yl derivative) ethers;

either (b1) separating the (+) and (-) ethers so obtained by selective crystallization in a hydroalcoholic medium

or (b2) desacetylating the (+) and (-) ethers so obtained and separating the resultant (+) and (-) desacetylated ethers by selective crystallization in a hydroalcoholic medium; and

(c) breaking the ether bond between the monosaccharide and the furo[3,4-c]pyridine derivative.

The desacetylation should be carried out before the selective crystallization, that is option (b2) should be followed, when the desacetylated ethers show a greater difference of solubility in the hydroalcoholic medium than the acetylated. If option (b1) is followed, the separated (+) and (-) ethers may be desacetylated before proceeding to step (c).

The hydroalcoholic medium usually comprises an aqueous solution of a methanol, ethanol or a propanol.

The term monosaccharide is intended to include a glucuronate ester or a glycoside. The term O-acetylated monosaccharide halogenide generally means a bromide or a chloride of the said monosaccharide, wherein all the hydroxy groups are acetylated. If the monosaccharide halogenide is a

glucuronate ester, the ester function in the separated (+) and (-) ethers may be hydrolysed before proceeding to step (c).

The following examples illustrate the invention.

EXAMPLE 1

This example describes the separation of the stereoisomers of 1,3-dihydro-6-methyl-3-(p-chloro)phenyl-7-hydroxy--furo[3,4-c]pyridine.

(I : R_3 = Cl , R_1 = H, R_4 = CH_3).

Reaction No.1

Synthesis of (+)-1,3-dihydro-6-methyl-3-[-chlorophenyl--furo[3,4-c]-pyridine 7-0-(β-D-glucuronate) methyl ester triacetate and (-)-1,3-dihydro-6-methyl-3-p-chlorophenyl--furo[3,4-c]pyridine 7-0-(β D-glucuronate) methyl ester triacetate.

4.06 g (0.015 mole) of 1,3-dihydro-6-methyl-3-p-chlorophenyl-7-hydroxy-furo[3,4-c]pyridine free base, 1.13 g (4 mmole) of mercuric chloride and 6.75 g of molecular sieve (4 Å) were suspended in 85 ml of 1,2-dichloroethane, and refluxed for 15 minutes. 8.18 g (0.020 mole) of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate was then added in one portion, and refluxing was continued under nitrogen for 15 hours. After cooling, the solvent was decanted into a separatory funnel, and the flask/sieves were rinsed five times with 50 ml aliquots of methylene dichloride. The organic layers were combined, and extracted 3 times with 75 ml aliquots of 10% potassium iodide solution, 3 times with some aliquots of 5% sodium bicarbonate solution and twice with 30 ml aliquots of saturated sodium chloride solution. After drying on

anhydrous magnesium sulphate, the solution was filtered and the filtrate was stripped to dryness on a rotary evaporator. The crude residue (7.5 g) was dissolved in 150 ml of methanol. On standing at room temperature, two crops of a fluffy white solid (2.26 g) were collected. The filtrate was concentrated to 20 ml, and provided on cooling a second solid which was recrystallized from methanol. Filtration provided 1.6 g of a crystalline white solid (yield = 44.5%).

Based on spectroscopic evaluation, the first solid was determined to be the (-)methyl ester tri-acetate and the second solid the (+)methyl ester tri-acetate.

Analysis for (+)compound calculated for $C_{27}H_{28}Cl\ N\ O_{11}$, C = 56.11%, H=4.88%, N=2.42%, observed: C=55.90%, H=4.80%, N=2.64%, ^{1}H NMR in $CDCl_3$, 2.03 ppm (3H, s), 2.05 ppm (3H, s), 2.13 ppm (3H, s), 2.49 ppm (3H, s), 3.75 ppm (3H, s); ^{13}C NMR in $CDCl_3$, 18.7 ppm (CH_3), 20.42 ppm (CH_3), 52.77 ppm (O-CH_3), 101.0 ppm (C-1'); (C=0.41 in $CHCl_3$); HPLC, R_T=10.88 min. (98.0%), m.p. 230-231°C.

Reaction No. 2

Synthesis of desacetylated form of (-)methyl ester compound of Reaction 1

0.5 g (0.8 mmole) of the (-)methyl ester tri-acetate obtained in Reaction No. 1 was dissolved in 5 ml of 1.5N methanolic hydrogen chloride. The solution was placed in a 25 ml round bottomed flask fitted with a condenser and nitrogen flask. The HPLC chromatographic profile in the

selected solvent system was established at time t = 0 by aliquot sampling. The flask was then placed in an oil bath at 85°C, and the rate of hydrolysis was followed at 10 minutes, 30 minutes, and 45 minutes. By the time all but 2.1% of starting material had been converted to a more polar product (R_T=4.58 minutes) which represented 75.6% of the mixture. 2 ml of water was added and the methanol was removed by rotary evaporation at room temperature. The aqueous solution was neutralized with 2 ml of 5% sodium bicarbonate solution. A white precipitate formed and was filtered off. Crystallization of the solid from ethanol yielded (76.7%) a fluffy white solid (280 mg).

Analysis: ^{1}H NMR in CD_3OD 2.54 ppm (3H, s), 3.77 ppm (3H, s), 7.3 ppm (4H, s), HPLC R_T= 4.44 min. (77.5%) m.p. +300°C.

Reaction No. 3

Hydrolysis of (-)methyl ester of Reaction No. 2

0.18 g (0.4 mmole) of the methyl ester obtained in Reaction No. 2 was dissolved in 1 ml of 2N hydrochloric acid in a reacti-vial, which was then sealed under nitrogen, and heated at 86°C. The rate of hydrolysis was followed by HPLC at 30 minutes, 1 hour and 18 hours. After this time, most (2.5% remaining) of the methyl ester had been converted to a major (91.2%) product eluting at R_T=5.17 minutes. The solution was adjusted to a pH of approximately 7 by addition of 5% sodium bicarbonate solution, and the solvent was removed by lyophilization. The crude solid was rinsed several times with ice cold methanol, and then filtered. The filtrate was evaporated to dryness. The solid residue was crystallised from methanol:water (1:1 by volume) and collected as a white solid (150 mg, 85.2% yield).

Analysis: ^{1}H NMR in CD_3OD 2.50 ppm (3H, s), 3.48 ppm = (3H, m), 5.33 ppm (2H, q, J=13 Hz), 7.28 ppm (4H, s), ^{13}C NMR in CD_3OD 19.39 ppm (CH_3), 104.9 ppm (C-1'), M.S. m^+/z 261, HPLC R_T=12.78 min. (98.5%), m.p. 247-248°C.

Reaction No. 4

Synthesis of (-)-1,3-dihydro-6-methyl-3-p-chlorophenyl-7--hydroxy-furo[3,4-c]pyridine

0.14 g (0.4 mmole) of the compound obtained in Reaction No. 3 was placed in a 25 ml round bottomed flask fitted with a condensor and nitrogen flush. The solid was dissolved in 1 ml of 6N hydrochloric acid. The solution was heated at 85°C for 1½ hours, and then cooled in an ice bath. 5% sodium bicarbonate solution was added to neutrality. The heterogeneous mixture was stripped to dryness on a rotary evaporator, and the solid residue was rinsed several times with methanol. After removing most of the methanol, the solution was spotted onto a 2000 μ silica prep TLC plate and eluted with dichloromethane : methanol (5:1 by volume). The major UV band at Rf=0.65 was removed. The organic material was rinsed free of silica with methanol, and the filtrate was stripped to dryness.

The recovered white solid (0.1 g, 96%) was found to co-elute with authentic 1,3-dihydro-6-methyl-3-p-chlorophenyl-7-hydroxy-furo[3,4-c]pyridine by HPLC.

Analysis: ^{1}H NMR in $CDCl_3/CD_3OD$ 2.46 ppm (3H, s), 5.21 ppm (2H, q, J=12Hz), 6.16 ppm (1H, s), 7.31 ppm (4H, s), 7.56 ppm (1H, s), $[\delta]_D^{26}$ = -109.4° (C=73 in methanol).

Reaction No. 5

Synthesis of desacetylated form of (+)methyl ester compound of Reaction 1

0.5g (0.8 mmole) of the (+)methyl ester tri-acetate obtained in Reaction No. 1 was dissolved in 5 ml of 1.5 N

methanolic hydrogen chloride. The solution was placed in a 25 ml round bottomed flask fitted with a condensor and nitrogen flask. The HPLC chromatographic profile in the selected solvent system was established at time t=0 by aliquot sampling. The flask was then placed in an oil bath at 85°C, and the rate of hydrolysis was followd at t=20 minutes, 40 minutes and 60 minutes. Total consumption of the starting material was observed in this time, and a major product (86.7%) was found at R_T=5.74 minutes. 2 ml of water was added and the methanol was removed by rotary evaporation at room temperature. The aqueous solution was neutralised with 2 ml of 5% sodium bicarbonate solution. The white solid (340 mg) which formed was collected (yield = 93.2%).

Analysis: ^{1}H NMR in $CD_3OD/CDCl_3$, 2.54 ppm (3H s), 3.76 ppm (3H, s), 5.40 ppm (2H, q, J=102 Hz), 7.29 ppm (4H, s).

Reaction No. 6

Hydrolysis of the (+)-methyl ester of Reaction 5

Without purification, the whole (340 mg, 0.74 mmole) of the product obtained in Reaction No. 5 was placed in a reacti-vial with 1 ml of 2N hydrochloric acid. The reacti-vial was sealed under nitrogen, and heated at 85°C for 18 hours. The solution was then cooled and 5% sodium bicarbonate solution was added to adjust the pH to approximately 7. The solvent was removed by lyophilization. The crude solid was rinsed several times with ice cold methanol, and was then filtered. The filtrate was stripped to dryness. The solid residue was crystallised from methanol:water 4:1 by volume, and the resultant white solid (180 mg, 58.4% yield) was collected.

Analysis: ^{1}H NMR in CD_3OD 2.56 ppm (3H, s), 3.55 ppm (3H, m), 5.48 ppm (2H, s), 6.16 ppm (1H, s), 7.34 ppm (4H, s);

^{13}C NMR in CD_3OD 19.56 ppm (CH_3), 105.21 ppm (C-1'); M.S. m^+/z 261; HPLC m.p. 259-261°C.

Reaction No. 7

Synthesis of (+)-1,3-dihydro-6-methyl-3-p-chlorophenyl-7--hydroxy-furo[3,4-c]pyridine

0.17 g (0.38 mmole) of the compound obtained in Reaction No. 6 was placed in a 10 ml round bottomed flask fitted with a condensor and nitrogen flask. The solid was dissolved in 1 ml of 6N hydrochloric acid. The solution was heated at 85°C for 1½ hours, and then cooled in an ice bath. 5% sodium bicarbonate solution was added until neutrality. The heterogeneous mixture was stripped to dryness on a rotary evacuator, and the solid residue was rinsed several times with methanol. After removing most of the methanol, the solution was spotted onto a 2000 μ silica prep TLC plate and eluted with dichloromethane : methanol (5:1 by volume). The major U.V. band at Rf = 0.65 was removed. The organic material was rinsed free of silica with methanol, and the filtrate was stripped to dryness. The recovered white solid (70 mg, 70.5% yield) co-eluted with authentic 1,3-dihydro-6-methyl-3-p-chloro-methyl-7-hydroxy-furo[3,4-c]pyridine on TLC and had an identical retention time to authentic 1,3-dihydro-6--methyl-3-p-chlorophenyl-7-hydroxy-furo[3,4-c]pyridine by HPLC.

Analysis: $[\delta]_D^{26}$ = +117.3° (C=4.67 in methanol).

EXAMPLE 2

This example describes the separation of the stereoisomers of 1,3-dihydro-6-methyl-3-isopropyl-7--hydroxy-furo[3,4-c]pyridine (I: $R_3=(CH_3)_2CH$, $R_4=H$, $R_6=CH_3$) with (D-tetraacetateglucosyl)bromide.

Reaction No. 1

Synthesis of (+) and (-)-1,3-dihydro-6-methyl-3-isopropyl-furo[3,4-c]pyridine 7-O-(D-tetraacetate-glucosyl)

The reaction of (tetra-O-acetyl-glucosyl) bromide on 1,3-dihydro-6-methyl-3-isopropyl-7-hydroxy-furo[3,4-c] pyridine, was carried out under the same conditions as in Reaction No. 1 of Example 1. Recrystallization was however not effected because the difference in solubility of the (+) and the (-) forms of the resulting compound in alcoholic media is not sufficient.

Reaction No. 2

Desacetylation and separation of the (+) and the (-) 1,3-dihydro-6-methyl-3-isopropyl-furo[3,4-c]pyridine 7-O-(D-tetraacetate-glucosyl)

Into a 100 ml flask (Erlen), there were poured 6 g (12.4 mmole) of the 1,3-dihydro-6-methyl-3-isopropyl--furo [3,4-c]pyridine-7-O-(D-tetraacetate-glucosyl) obtained in Reaction No.1 and 50 ml of methanol. The mixture was cooled with ice to 10°C, and then saturated with ammonia. The mixture was allowed to rest overnight in a refrigerator. After filtration, the precipitate obtained was washed with methanol, and then with diethyl-ether. The resulting compound was treated with 160 ml of an ethanol:water mixture (50:50 by volume). 1.3 g of white solid were filtered off. The filtrate was concentrated, and provided a second solid, which was recrystallised in ethanol. After filtration, there was obtained 1.4 g of a crystalline solid (overall yield : 67%).

Based on spectroscopic valuation, the first solid was determined to be the (-) and the second solid the (+) 1,3-dihydro-6-methyl-3-isopropyl-7-O-(D-glucosyl)-furo [3,4-c]pyridine.

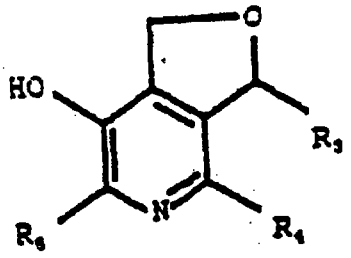
Reaction No. 3

Synthesis of the (+)-1,3-dihydro-6-methyl-3-isopropyl-7--hydroxy-furo[3,4-c]pyridine

Into a 50 ml flask (Erlen), there were poured 0.57 g (1.6 mmole) of the (+) compound obtained in the above Reaction No. 2, 3 ml of 6N hydrochloric acid and 1 ml of water. The mixture was warmed to 80°C for about two hours, and cooled to room temperature. The precipitate obtained was filtered off, and washed with water. Dissolution of the precipitate occurs. The wash waters were neutralized with sodium bicarbonate, and precipitation occurred. The precipitate was then washed with water and dried to yield 0.20 g (65%) of the title compound.

CLAIMS

1. A method for the separation of stereoisomers of 7-hydroxy-furo[3,4]pyridine derivatives of the general formula I



I

wherein R_3 represents a hydrogen atom, a straight chain saturated hydrocarbon group having from 1 to 5 carbon atoms, an unsaturated hydrocarbon group having from 2 to 5 carbon atoms, a heterocyclic group having up to 6 ring atoms, a phenyl group, a phenylalkyl or phenylalkenyl group, said groups being optionally substituted by one or more chlorine or fluorine atoms, trifluoromethyl groups, alkyl groups having from 1 to 5 carbon atoms, alkoxy groups having from 1 to 5 carbon atoms, alkylthio groups having from 1 to 5 carbon atoms, dialkylamino groups in which each alkyl group has from 1 to 5 carbon atoms, dialkylaminoalkoxy groups in which each of the two alkyl groups and the alkoxy group has from 1 to 5 carbon atoms or α or ß-alkoxy-N-pyrrolidinyl groups in which the alkoxy group has from 1 to 5 carbon atoms; R_4 represents a hydrogen or chlorine atom and R_6 represents a saturated or unsaturated hydrocarbon group up to C_5, optionally substituted by a hydroxy radical, by an amino residue of the formula $N(R)_2$ wherein R represents a hydrogen atom or an alkyl group up to C_3,

which method comprises:

(a) reacting a fully O-acetylated monosaccharide halogenide with a racemate of the selected 7-hydroxyfuro[3,4-c]pyridine derivative to form the corresponding (+) and (-) (O-acetylated monosaccharide) (furo[3,4-c]pyridine-7-yl derivative) ethers;

either (b1) separating the (+) and (-) ethers so obtained by selective crystallization in a hydroalcoholic medium

* C L M S *

or (b2) desacetylating the (+) and (-) ethers so obtained and separating the resultant (+) and (-) desacetylated ethers by selective crystallization in a hydroalcoholic medium; and

(c) breaking the ether bond between the monosaccharide and the furo[3,4-c]pyridine derivative.

2. A method according to claim 1, option (b1), further comprising desacetylating the separated (+) and (-) ethers before proceeding to step (c).

3. A method according to claim 1 or claim 2 in which the selective crystallization is performed in an aqueous solution of methanol, ethanol or a propanol.

4. A method according to any preceding claim in which the monosaccharide halogenide is a chloride or a bromide of a glycoside.

5. A method according to any of claims 1 to 3 in which the monosaccharide halogenide is a chloride or a bromide of a glucuronate-ester.

6. A method according to claim 5 in which the ester function in the separated derivatives is hydrolysed before proceeding to step (c).

7. A method for the separation of stereoisomers as herein described in Example 1.

8. A method for the separation of stereoisomers as herein described in Example 2.

9. A stereoisomer separated by a method according to any preceding claim.

* C L M S *